We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Solid-State Fermentation in a Bag Bioreactor: Effect of Corn Cob Mixed with Phytopathogen Biomass on Spore and Cellulase Production by *Trichoderma asperellum*

Reynaldo De la Cruz-Quiroz, Sevastianos Roussos, Daniel Hernandez-Castillo, Raúl Rodríguez-Herrera, Lluvia López-López, Francisco Castillo and Cristóbal N. Aguilar

Additional information is available at the end of the chapter

Abstract

The solid-state fermentation (SSF) is the best option to produce spores of biological control agents (BCA), because the spores have a long shelf life, compared with the obtained in liquid cultures. The spore production under SSF conditions using polyethylene bioreactors (bag-type) is a new topic. Only little information mainly about bioreactors design and adequate conditions to spore production is available. The main aim of this study was to use the corn cob as substrate in SSF and produce spores of the fungi BCA *Trichoderma asperellum* in a polyethylene bioreactor. In the process was added biomass of the phytopathogenic fungi *Colletotrichum gloeosporioides* and *Phytophthora capsici* as inducers of hydrolase enzymes (endoglucanases, exoglucanases and chitinases). It is possible to obtain high levels of spores, cellulases and chitinases using a polyethylene bioreactor under SSF conditions by *T. asperellum* and corn cob as substrate. Under the SSF conditions evaluated, the biomass of *C. gloeosporioides* has an inducer effect just on the spore production. However, *P. capsici* have effect on all response variables evaluated. The spore production was twice when used *P. capsici* as inducer. The most influential factor under SSF was the moisture. Levels of 66 and 50% of this factor increase the yield in all response variables evaluated (sporulation, cellulases and chitinases), *C. gloeosporioides* and *P. capsici*, respectively.

Keywords: spores, cellulase, *Trichoderma asperellum*, solid-state fermentation, bag bioreactor

1. Introduction

The diseases induced by phytopathogens are the leading cause of losses in the most crops worldwide. It is well known that the control of such diseases through the use of chemical pesticides is not effective, they generate resistant strains phytopathogenic, the wastes are toxic and they have carcinogenic effects [1, 2]. In the last years, the alternative proposed is the use of antagonist microorganism of phytopathogens, which results in an adequate biological control, which is highly effective and environmental friendly [2]. For this, the production of high concentrations of the spores of biological control agents (BCA) is necessary, and so currently there are several production processes of different microorganisms. The solid-state fermentation (SSF) is the best to this aim, because the spores have a long shelf life, compared with the obtained in liquid cultures [3, 4]. In other way, the spore production in SSF is relatively easiest, so it can be realized by personal with no experience, and therefore make possible the technology transference to farmers [3]. The spore production under SSF conditions using polyethylene bioreactors (bag-type) is a new topic. Only little information mainly about bioreactors design and adequate conditions to spore production [1, 5, 6] is available. In SSF, most of the time wastes from other manufacturing process are used; therefore, this potential is commonly investigated in developing countries [1]. Wastes of rice, maize meal, corn cob, rice husk, banana husk, wheat bran and tea leaves, among others have been used as substrate to spore production by SSF [3, 5, 6]. There are some compounds that can be added to the substrate of SSF in little proportions to induce some interest metabolite. For example, there are reports of the addition of casein and gluten to produce proteases, waste shrimp silage to chitinase production, among others [7, 8]. The main aim of this study was to use the corn cob as substrate in SSF and produce spores of the fungi BCA *Trichoderma asperellum* in a polyethylene bioreactor. In the process was added biomass of the phytopathogenic fungi *Colletotrichum gloeosporioides* and *Phytophthora capsici* as inducers of hydrolase enzymes (endoglucanases, exoglucanases and chitinases).

2. Materials and methods

2.1. Microorganism and culture conditions

The *T. asperellum* (T2-10) and *P. capsici* were kindly proportioned by the Agricultural Parasitology Department of the UAAAN (Universidad Autónoma Agraria Antonio Narro, Saltillo, México). *C. gloeosporioides* was proportioned by the Food Research Department of the UAdeC (Universidad Autónoma de Coahuila). The fungi were cultivated and conserved in a milk-glycerol 8.5% solution. Potato dextrose agar (PDA) was used to reactivate the fungi. In HACH® tubes, 5 mL of PDA was taken, then they were closed and sterilized at 121°C for 15 min. The tubes in slant were inoculated with the fungal strains and incubated at 30°C for 5 days. The conservation was at ± 4°C.

2.2. Phytopathogen biomass production

A cornmeal medium (17 g/L) was used to produce phytopathogen biomass. This medium was maintained under shaking for 1 h at 58°C. Then, it was filtrated and sterilized (15 at 115°C). The inoculation of phytopathogens was as follows: *C. gloeosporioides* ($1×10^6$ spores/mL) and *P. capsici* (10 PDA plugs from a culture of 7days old), and incubated at 28°C for 7 days under shaking (200 rpm).

2.3. Substrates

In this work, we evaluate as substrate corn cob (CC) proportioned by the Mexican Institute of Maize, UAAAN Coahuila, México. The material was dried, ground, fractioned (300–1680 μm) and stored under low moisture conditions for further evaluation. This material was used as a substrate on SSF without pretreatment.

2.4. Solid-state fermentation

Polyethylene bags were used as bioreactor in all experiments. Sporulation and cellulase production were evaluated. Plackett-Burman design (PBD) was used in this experiment to determine the most influential factors on spore and enzyme production by *T. asperellum* under SSF conditions on a bag bioreactor. The factors such as temperature (°C), pH, substrate (g), inoculum (spores/g), moisture (%), phytopathogen biomass (%) and incubation time (days) were evaluated, one maximum (+1) and one minimum (−1) (**Table 1**). Spore counting was done at the end of SSF process using a hemocytometer. The fermented material was placed in a Falcon® tube with 10 mL of distilled water. The enzymatic extract was homogenized in a vortex (1 min) for further determination of enzyme activity.

2.5. Enzyme activity determination

After SSF each sample was analyzed to determine cellulase activity [9], chitinase activity [10] and reducing sugars [11]. The carboxymethylcellulose activity (CMCA) was carried out at 50°C for 30 min. Sample (1 mL) and substrate (1 mL of carboxymethylcellulose 1%) were the mix reaction. Citrate buffer (1 mL at 50 mM, pH 4.8) and substrate (1 mL) were the substrate control. The enzyme control was the mix of sample (1 mL) and citrate buffer (1 mL).

The filter paper activity (FPA) was carried out at 50°C for 1 h. Sample (1 mL) and substrate (filter paper Whatman No.1 (1 cm×5 cm) in 1 mL of citrate buffer at 50 mM, pH 4.8) were the reaction mix. The control substrate was the mix of citrate buffer (2 mL) and filter paper. Sample (1 mL) and citrate buffer (1 mL) were the enzyme control.

Chitinase activity was carried out at 37°C for 1 h. The reaction mix, enzyme and substrate control were done similar to carboxymethylcellulose activity. In this case, substrate (chitin oligosaccharides) and buffer solution (acetate 50 mM, pH 4.0) were replaced.

Sugar concentration was determined after each enzyme reaction. An enzyme activity (U) was defined as the amount of enzyme that catalyze the release of 1 μmol of glucose per minute.

Run	A	B	C	D	E	F	G
1	−1	−1	−1	1	1	1	−1
2	1	−1	−1	−1	−1	1	1
3	−1	1	−1	−1	1	−1	1
4	1	1	−1	1	−1	−1	−1
5	−1	−1	1	1	−1	−1	1
6	1	−1	1	−1	1	−1	−1
7	−1	1	1	−1	−1	1	−1
8	1	1	1	1	1	1	1

Code	Factors	High value	Low value
A	Substrate (g)	30	15
B	pH	8.0	6
C	Inoculum (spores/g)	1×10^7	1×10^5
D	Temperature (°C)	30	24
E	Moisture (%)	66	50
F	Inducer (%)	3	1
G	Time (days)	7	5

Table 1. PBD matrix used to determine the influence of different variables (A, B, C, D, E, F and G) on spore and enzyme activity in SSF by *T. asperellum*.

2.6. Design and statistical analysis

A PBD was used to SSF. Spore and enzyme production were the response variables. Data were analyzed by ANOVA using STATISTICA 7.0 software; when needed mean treatments were compared using Tukey's multiple range procedure. A *p*-value of less than 0.05 was regarded as significantly different.

3. Results

3.1. Screening of significant factors using Plackett-Burman design

Studies were performed in eight runs each one to identify the combination of factors which allow us to obtain a significant level of spore, cellulase and chitinase production by *T. asperellum* on corn cob under SSF conditions using phytopathogen biomass (*C. gloeosporioides* and *P. capsici*).

3.2. Solid-state fermentation with C. gloeosporioides biomass

Table 2 summarizes the results obtained in SSF using the biomass of *C. gloeosporioides* blended with corn cob. The sporulation index is favored by the treatments F and G (7.3×10^8 and 6.2×10^8 Spores/g CS, respectively), with no significant difference among the values. The conditions of treatment C allow the best production to CMCA, FPA and CA (2.582, 1.549 y 5.118 U/g), respectively).

Treatment	Sporulation index (Spores/g)	Enzyme activities		
		CMCA	FPA	CA
A	4.2E+08 ± 6.25E+07[cd]	1.847 ± 0.02[b]	1.183 ± 0.17[b]	3.671 ± 0.46[b]
B	4.0E+08 ± 4.30E+07[d]	0.132 ± 0.02[d]	0.165 ± 0.06[cd]	1.795 ± 0.05[f]
C	4.8E+08 ± 5.10E+07[cd]	2.582 ± 0.08[a]	1.549 ± 0.05[a]	5.118 ± 0.28[a]
D	2.3E+08 ± 6.65E+07[e]	0.204 ± 0.10[d]	0.006 ± 0.00[d]	2.149 ± 0.06[ef]
E	2.3E+08 ± 7.35E+07[e]	0.529 ± 0.10[d]	0.443 ± 0.05[c]	2.996 ± 0.08[cd]
F	7.3E+08 ± 9.55E+07[a]	1.958 ± 0.17[b]	1.381 ± 0.23[ab]	3.692 ± 0.13[b]
G	6.2E+08 ± 8.15E+07[ab]	1.243 ± 0.02[c]	0.390 ± 0.01[c]	2.538 ± 0.01[de]
H	5.2E+08 ± 3.50E+06[bc]	2.474 ± 0.47[a]	1.193 ± 0.25[b]	3.418 ± 0.29[bc]

Numbers in each column followed by a common letter are not significantly different ($P<0.05$).

Table 2. Enzyme production and sporulation index of *T. asperellum* on a mixture of corn cob and *C. gloeosporioides* biomass under SSF conditions.
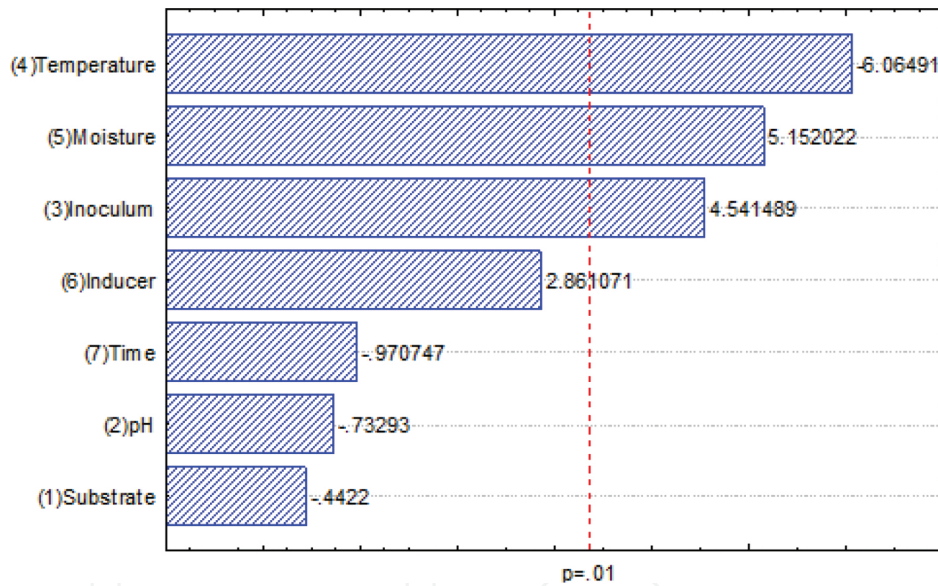

Figure 1. Pareto plot of the standardized effects on the spore production of *T. asperellum* using corn cob in SSF with *C. gloeosporioides* as inducer.

In the first case, the spore production was influenced by the temperature in a negative way. Between the range of the values (24 and 30°C), the study shows that 24°C is the best to produce a better sporulation index and possibly if we reduce the value, the sporulation can be major. The moisture, inoculum and inducer are the other factors that also have influence on spore production, just in a positive way. It means, it is necessary to increase the value of each factor (**Figure 1**). The moisture, pH and inoculum were the factors more determining endoglucanase production (CMCA). These factors had positive values, which mean that high values allow high enzyme activity. A significant effect was observed with the substrate concentration, but this effect was negative, so low amount of substrate is needed to obtain high enzyme yields (**Figure 2**).
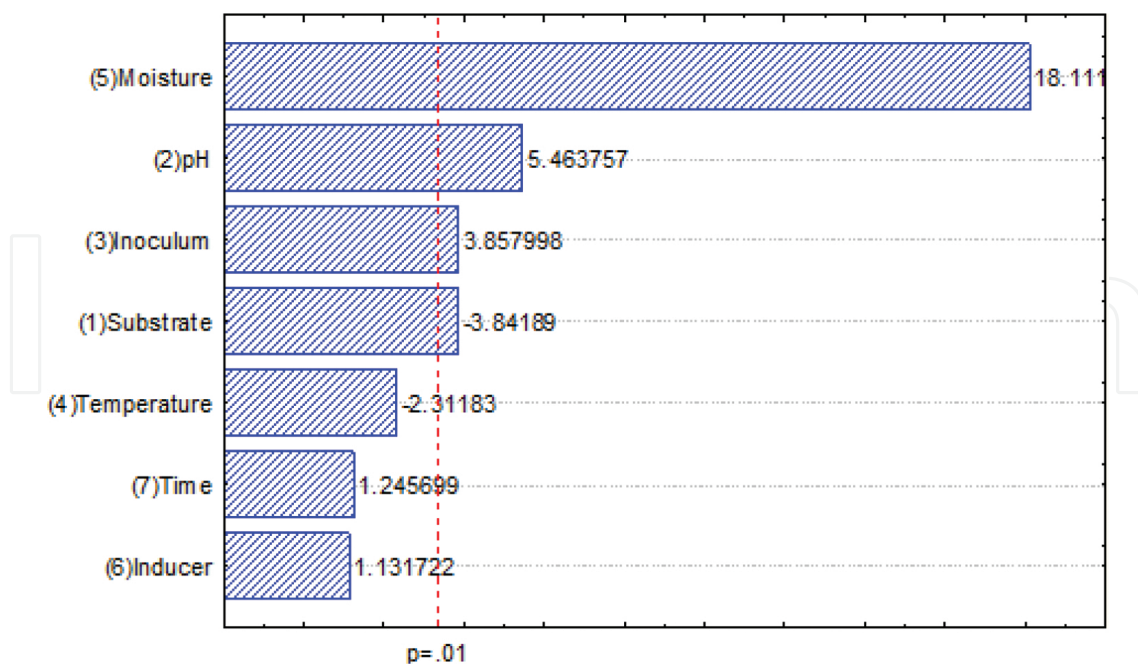
Figure 2. Pareto plot of the standardized effects on CMCA from an extract of *T. asperellum* using corn cob in SSF with *C. gloeosporioides* as inducer.
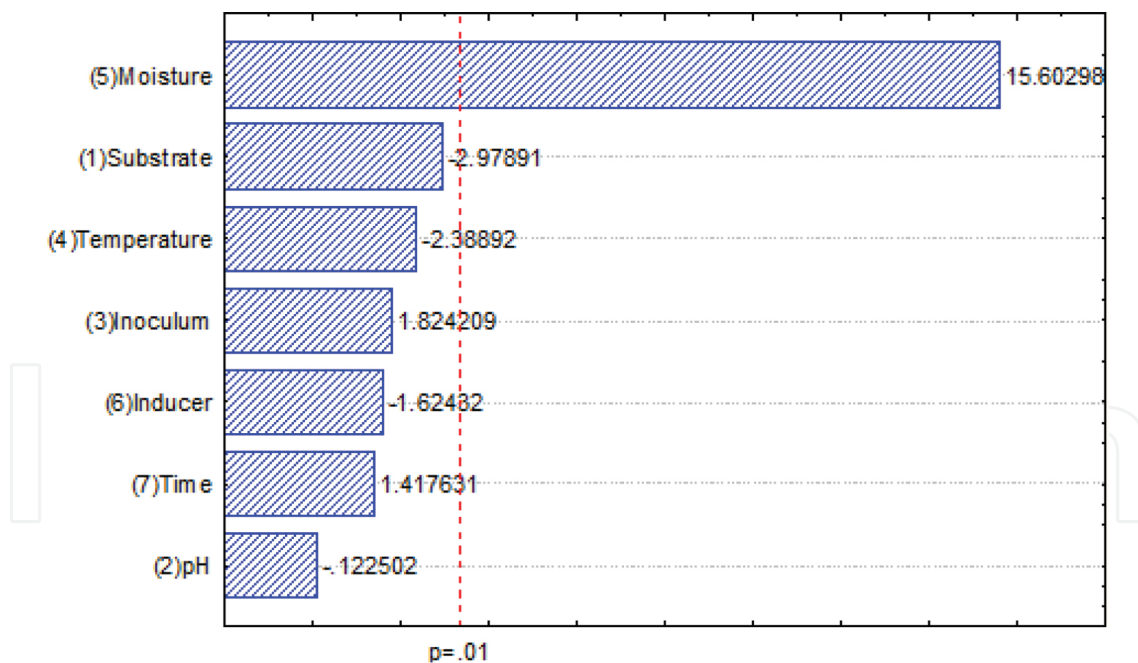

Figure 3. Pareto plot of the standardized effects on FPA from an extract of *T. asperellum* using corn cob in SSF with *C. gloeosporioides* as inducer.

The exoglucanase (FPS) in the same way to CMCA was influenced by the moisture (Positive). Low levels in the substrate and temperature show the best enzymatic yields (**Figure 3**). The moisture was the factor with major influence on the chitinase production. The substrate and

inducer were also significant, but in negative way, it is necessary to use low values to increase the yield. The time and pH were important, so these factors must be in high levels (**Figure 4**).
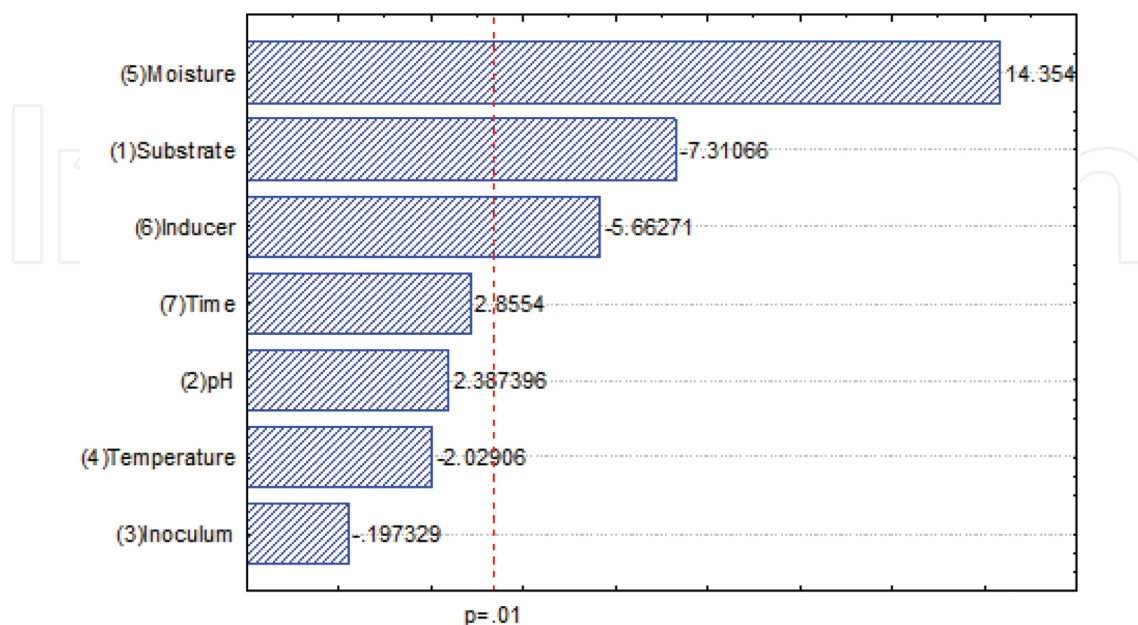

Figure 4. Pareto plot of the standardized effects on chitinase from an extract of *T. asperellum* using corn cob in SSF with *C. gloeosporioides* as inducer.

3.3. Solid-state fermentation with P. capsici as inducer

Now, **Table 3** shows the results obtained in SSF using the biomass of *P. capsici* as inducer. The conditions of treatment G allow the best production to all dependent variables evaluated. The values obtained were sporulation index ($1.2×10^9$ Spores/g CS), CMCA (7.825 U/g), FPA (2.764 U/g) and CA (3.609 U/g).

Treatment	Sporulation index (Spores/g)	Enzyme activities		
		CMCA	FPA	CA
A	3.1E+08 ± 2.0E+06[d]	4.238 ± 0.32[c]	1.660 ± 0.02[d]	2.848 ± 0.22[b]
B	1.4E+09 ± 1.5E+08[a]	4.861 ± 0.13[bc]	2.466 ± 0.14[b]	3.216 ± 0.15[ab]
C	2.6E+07 ± 1.0E+06[f]	0.062 ± 0.02[e]	0.121 ± 0.00[g]	1.527 ± 0.25[d]
D	8.1E+08 ± 9.0E+06[b]	5.012 ± 0.40[b]	2.272 ± 0.01[c]	2.978 ± 0.10[b]
E	4.4E+08 ± 6.0E+07[c]	4.814 ± 0.29[bc]	2.200 ± 0.02[c]	3.082 ± 0.04[b]
F	3.7E+08 ± 3.0E+07[d]	1.538 ± 0.56[d]	0.696 ± 0.01[e]	2.144 ± 0.24[c]
G	1.2E+09 ± 1.1E+08[a]	7.825 ± 0.21[a]	2.764 ± 0.01[a]	3.609 ± 0.18[a]
H	1.1E+08 ± 3.0E+06[e]	1.168 ± 0.21[d]	0.310 ± 0.13[f]	1.956 ± 0.22[cd]

Numbers in each column followed by a common letter are not significantly different ($P<0.05$).

Table 3. Enzyme production and sporulation index of *T. asperellum* grown on a mixture of corn cob with *P. capsici* biomass under SSF conditions.
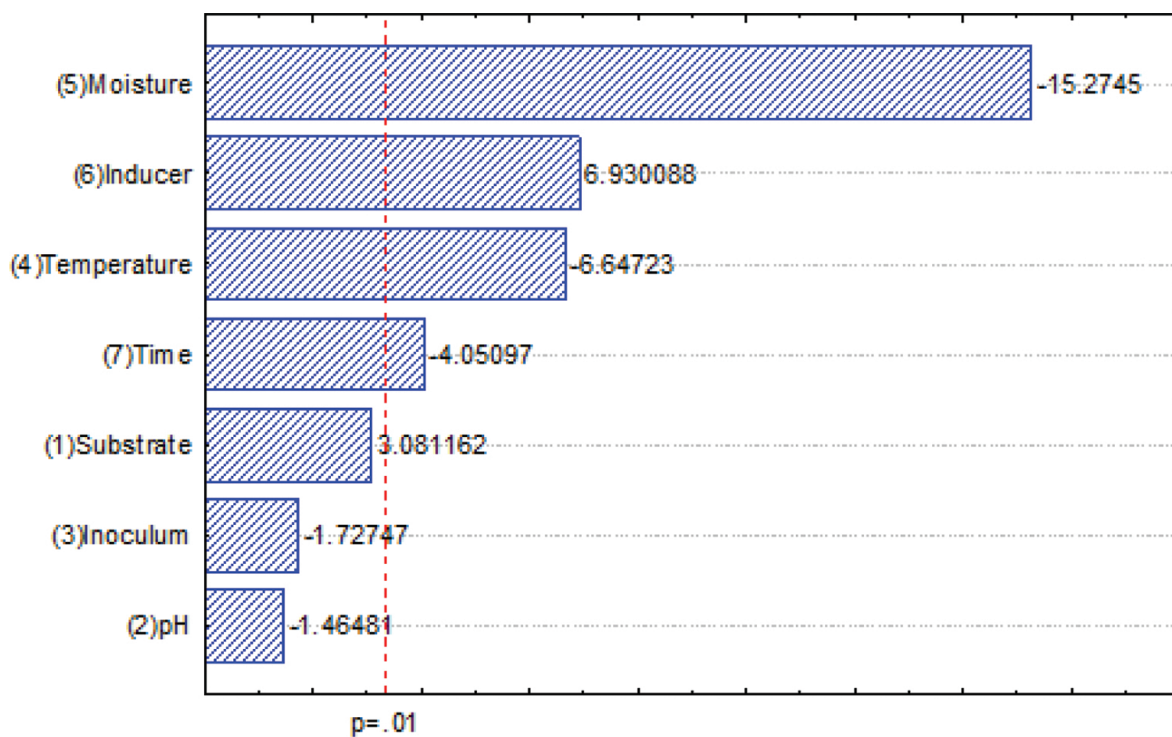
Figure 5. Pareto plot of the standardized effects on the spore production of *T. asperellum* using corn cob in SSF with *P. capsici* as inducer.
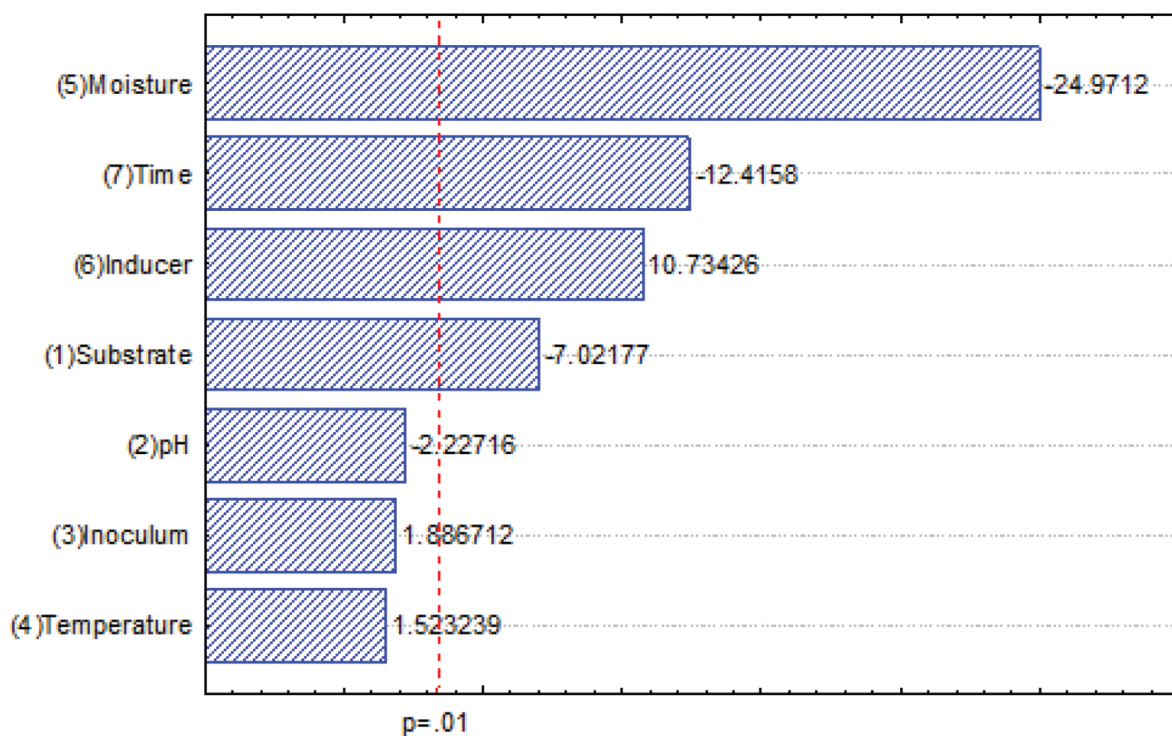

Figure 6. Pareto plot of the standardized effects on CMCA from an extract of *T. asperellum* using corn cob in SSF with *P. capsici* as inducer.
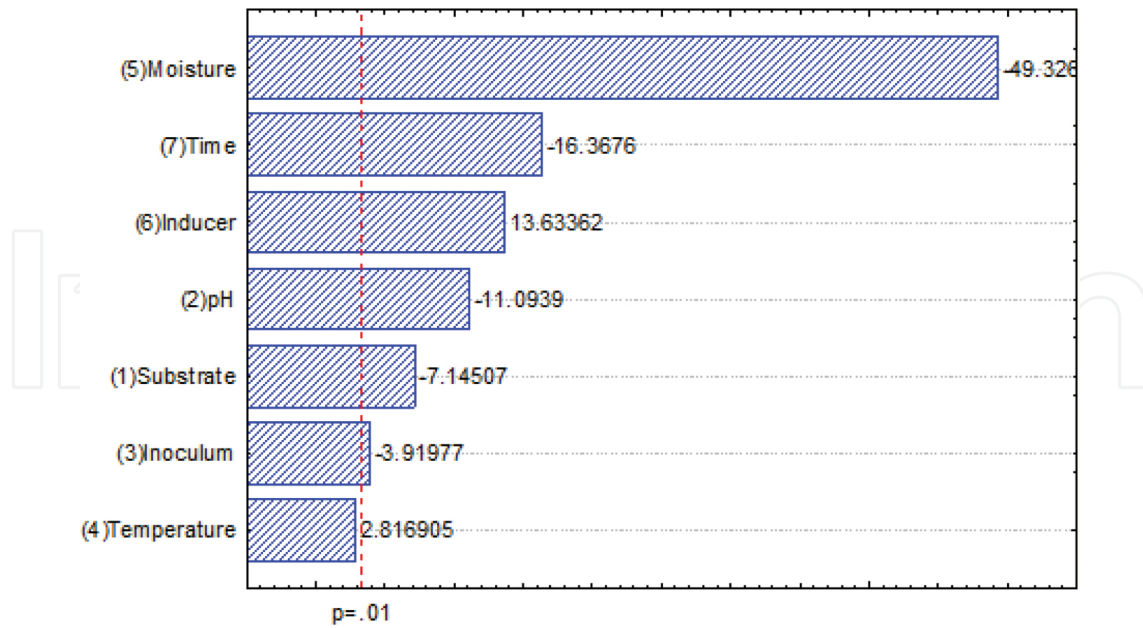
Figure 7. Pareto plot of the standardized effects on FPA from an extract of *T. asperellum* using corn cob in SSF with *P. capsici* as inducer.
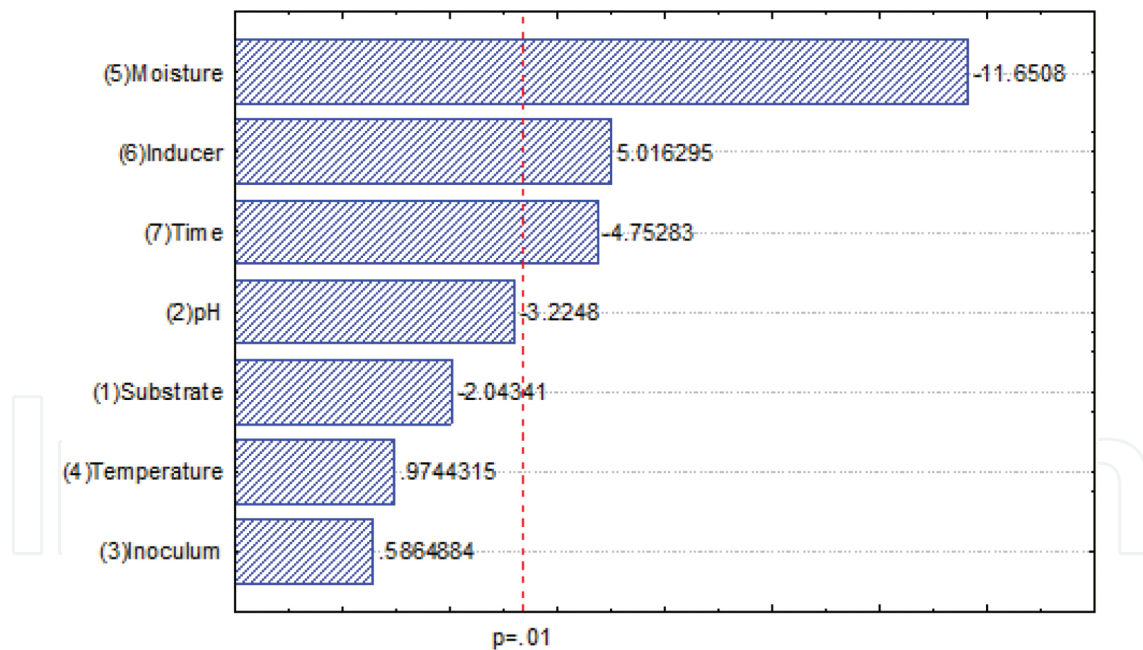

Figure 8. Pareto plot of the standardized effects on chitinase from an extract of *T. asperellum* using corn cob in SSF with *P. capsici* as inducer.

The sporulation of *T. asperellum* was not influenced by the pH and inoculum. But low levels (moisture, temperature and time) and high levels (inducer and substrate) show high spore production (**Figure 5**). In endoglucanase production, four factors are important under the SSF conditions evaluated. Low levels of moisture, time and substrate and high levels of the inducer

show the major levels of enzymatic activity (**Figure 6**). All factors evaluated were significant to the exoglucanase production. Low levels of moisture, time, pH, substrate ad inoculum and high levels of inducer and temperature show the best enzyme yields (**Figure 7**). Finally, low levels of moisture, time and pH and high levels of inducer shows the major chitinolytic activity (**Figure 8**).

4. Discussion

There are several studies that report the production of different enzymes under SSF [12, 13]. Currently, the SSF is a commonly used system because the raw materials such as sugarcane bagasse, wheat bran, among others [14] are cheaper. The control of temperature, pH, moisture, purity of the culture and process time are some factors that difficult the rigorous control of the fermentation process [8].

Sometimes, it is hard to find one combination of the SSF conditions in which we can obtain high yield in all response variables evaluated (sporulation, cellulases and chitinases). In the case of SSF with biomass of *C. gloeosporioides*, the best results were observed in the treatment F to spore production and the treatment C to enzyme activities. However, the treatment F also shows great enzyme yields. So the treatment F allow obtain high values of spores, cellulases and chitinases. Substrate (30 g), pH (6), inoculum (1×10^7 Spores/g), temperature (24°C), moisture (66%), inducer (1%) and time (5 days) were the treatment F conditions.

Now, in the SSF with *P. capsici* biomass, the best results were in the treatment G. It means that the spore, cellulase and chitinase production were high when the conditions are substrate (15 g), pH (8), inoculum (1×10^7 Spores/g), temperature (24°C), moisture (50%), inducer (3%) and 5 days of incubation.

In the start of the study, we think that the addition of certain concentration of phytopathogen biomass could generate an induction effect of some hydrolase enzymes. The production of chitinases when were used *C. gloeosporioides* and cellulases when were used *P. capsici*. This effect is influenced by the phytopathogen composition (chitin and cellulose, respectively).

In the SSF with *C. gloeosporioides*, the induction of enzymes did not happen maybe because the chitinase is a constitutive enzyme result of the natural metabolism of the microorganism. Previously, the same effect in the chitinase production with *Meyerozyma caribbica* in liquid culture using *C. gloeosporioides* as inducer [15] was observed. A similar study was also reported evaluating the β-*N*-acetylhexosaminidase (a chitinase) by *Verticillium lecanii* using shrimp waste silage as inducer and sugarcane bagasse as support [8].

In the case of SSF with *P. capsici*, the inductor was effective and shows an important effect in all enzyme activities evaluated. We did not find reports of the use of biomass to induce some types of cellulase.

In this study, the moisture and temperature are the two important factors. Among the values evaluated, a level of 66% of moisture and 24°C of temperature shows the best yields in spore

and enzymes production on SSF with *C. gloeosporioides* as inducer. In the SSF with *P. capsici* as inducer, the level of moisture was 50%. The two values of moisture used in this study are low which is reported in the literature [8]. They mentioned that the inducer is very important, but also the moisture because they observed that above 75% it can affect the porosity, oxygen diffusion and favor the bacterial contamination. In other hand, low moisture percentage reduces the microbial growth.

This study demonstrated that the biomass addition of any one phytopathogen shows an increment in the spore production by *T. asperellum*. The fungi sporulation starts when the environmental and nutritional conditions become hard to life support. The chemical composition of the inducer possibly causes some stress on *T. asperellum* which accelerate the sporulation process. The experimental stage suggests that high levels of biomass of the inducer increase the sporulation.

Currently, there researches are aimed at the high biomass production of the biological control agents using several systems to produce it. Kancelista *et al*. [16] reported the use of corn cob under SSF by *T. asperellum* obtaining a yield of 3.13×10^9 spores/g. Motta and Santana [17] who working the SSF with empty fruit bunch and a *Trichoderma* spp. The sporulation index was 4.4×10^9 spores/g in a Raimbault columns.

There are few works that report the use of polyethylene bioreactors to produce spores in SSF using some biological control. The use of this kind of bioreactor needs to utilize special plastic bags which allow the gas exchange and microorganism respiration [18]. In some cases, we can use a cotton tap on the bag to allow gas exchange. In this study, the maximal spore production obtained was 7.3×10^8 and 1.4×10^9 spores/g CS to the SSF with *C. gloeosporioides* and *P. capsici*, respectively. Singh *et al*. [6] used a *Trichoderma harzianum* strain and a similar bioreactor, obtaining a production of 8×10^8 y de 4.4×10^6 spores/g CS using tea leaves and sawdust, respectively. Viccini *et al*. [3] did a study of the spore production of *Clonostachys rosea* under SSF conditions using a polyethylene bioreactor and rice grains as substrate. The yield obtained was 1.8×10^8 spores/g CS.

5. Conclusion

It is possible to obtain high levels of spores, cellulases and chitinases using a polyethylene bioreactor under SSF conditions by *T. asperellum* and corn cob as substrate. Under the SSF conditions evaluated, the biomass of *C. gloeosporioides* has an inducer effect just on the spore production. However, *P. capsici* have effect on all response variables evaluated. The spore production was twice when used *P. capsici* as inducer. The most influential factor under SSF was the moisture. Levels of 66 and 50% of this factor increase the yield in all response variables evaluated (sporulation, cellulases and chitinases), *C. gloeosporioides* and *P. capsici*, respectively. When the biomass of *C. gloeosporioides* was used as a inducer, the best SSF conditions with corn cob and *T. asperellum* are as follows: substrate (30 g), pH (6), inoculum (1×10^7 Spores/g), temperature (24°C), moisture (66%), inducer (1%) and time (5 days). In the case of *P. capsici*, the conditions are: substrate (15 g), pH (8), inoculum (1×10^7 Spores/g), temperature (24°C),

moisture (50%), inducer (3%) and time (5 days). Further research on SSF with agroindustrial wastes using polyethylene bioreactors, mainly to the reduction of cost in the process, is necessary. Also, it must be make more analyses to determine the optimal production conditions, as well as, the use of inducers.

Acknowledgements

Author details

Reynaldo De la Cruz-Quiroz[1], Sevastianos Roussos[2], Daniel Hernandez-Castillo[3], Raúl Rodríguez-Herrera[1], Lluvia López-López[1], Francisco Castillo[4] and Cristóbal N. Aguilar[1*]

*Address all correspondence to: cristobal.aguilar@uadec.edu.mx

1 Group of Bioprocesses, Food Research Department, Universidad Autónoma de Coahuila, Blvd. V. Carranza esquina González Lobo, Colonia República Oriente, Saltillo, México

2 Equipe Eco technologies et Bioremédiation, Aix Marseille Université & Université Avignon; IMBE UMR CNRS-7263/IRD-237, Case 421, Campus Etoile, Faculté St Jérôme, Marseille Cedex 20, France

3 Agricultural Parasitology Department, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro, Buenavista, Saltillo, México

4 National Institute of Forestry, Agriculture and Livestock Research, Blvd. Vito Alessio Robles, Col. Nazario Ortiz Garza, Saltillo, México

References

[1] Millner RJ. 2000. Current status of *Metarhizium* as mycoinsecticide in Australia. *Biocontrol News and Information*. 21(2):47-50. doi:10.1080/03235400601160065.

[2] Abo-Elyousr KAM, Abdel-Hafez SII, Abdel-Rahim IR. 2014. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri. Journal of Phytopathology*. 162: 567–574. doi:10.1111/jph.12228.

[3]   Viccini G, Mannich M, Fontana-Capalbo DM, Valdebenito-Sanhueza R, Mitchell DA. 2007. Spore production in solid-state fermentation of rice by *Clonostachys rosea*, a biopesticide for gray mold of strawberries. *Process Biochemistry*. 42:275-278. doi:10.1016/j.procbio.2006.07.006.

[4]   Hanada RE, Pomella AWV, Soberanis W, Loguercio LL, Pereira JO. 2009. Biocontrol potential of *Trichoderma martiale* against the black-pod disease (*Phytophthora palmivora*) of cacao. *Biological Control*. 50:143-149. doi:10.1016/j.biocontrol.2009.04.005.

[5]   Singh HB, Singh BN, Singh SP, Nautiyal CS. 2010. Solid-state cultivation of *Trichoderma harzianum* NBRI-1055 for modulating natural antioxidants in soybean seed matrix. *Bioresource Technology*. 101:6444-6453. doi:10.1016/j.biortech.2010.03.057

[6]   Singh A, Srivastava S, Singh HB. 2007. Effect of substrates on growth and shelf life of *Trichoderma harzianum* and its use in biocontrol of diseases. *Bioresource Technology*. 98:470-473. doi:10.1016/j.biortech.2006.01.002.

[7]   Battaglino RA, Huergo M, Pilosof AMR, Bartholomai GB. 1991. Culture requirements for the production of protease by *Aspergillus oryzae* in solid state fermentation. *Applied Microbiology and Biotechnology*. 35:292-296. doi:10.1007/BF00172714.

[8]   Matsumoto Y, Saucedo-Castañeda G, Revah S, Shirai K. 2004. Production of β-*N*-acetylhexosaminidase of *Verticillium lecanii* by solid state and submerged fermentations utilizing shrimp waste silage as substrate and inducer. *Process Biochemistry*. 39:665-671. doi:10.1016/S0032-9592(03)00140-7

[9]   Galindo, A. I. G., Medina-Morales, M. A., Romanini, D., Pico, G., & Aguilar, C. N. (2016). Cellulase production by solid state fermentation and its separation with polyacrylate. *New Biotechnology*, 33(3):429.

[10] Saito, A., Fujii, T., Yoneyama, T., Redenbach, M., Ohno, T., Watanabe, T., & Miyashita, K. (1999). High-multiplicity of chitinase genes in *Streptomyces coelicolor* A3 (2). *Bioscience, Biotechnology, and Biochemistry*. 63(4):710-718. doi:10.1271/bbb.63.710

[11] Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 31(3):426-428. doi:10.1021/ac60147a030

[12] Buenrostro-Figueroa, J., Ascacio-Valdés, A., Sepúlveda, L., De la Cruz, R., Prado-Barragán, A., Aguilar-González, M. A., Rodriguez, R., Aguilar, C. N. 2014. Potential use of different agroindustrial by-products as supports for fungal ellagitannase production under solid-state fermentation. *Food and Bioproducts Processing*. 92(4): 376-382. doi:10.1016/j.fbp.2013.08.010

[13] Pirota RDPB, Delabona PS, Farinas CS. 2014. Simplification of the biomass to ethanol conversion process by using the whole medium of filamentous fungi cultivated under solid-state fermentation. *BioEnergy Research*. 7:744–752. doi:10.1007/s12155-013-9406-4.

[14] Shahzadi T, Ikram N, Rashid U, Afroz A, But HI, Anwar Z, Irshad M, Mahmood S. 2013. Optimization of physical and nutritional factors for induced production of

cellulase by co-culture solid-state bio-processing of corn stover. *WSEAS Transactions on Environment and Development*. 4(9):263-267. Available at: http://www.wseas.org/multimedia/journals/environme…

[15] Bautista-Rosales PU, Calderon-Santoyo M, Servín-Villegas R, Ochoa-Álvarez NA, Ragazzo-Sánchez JA. Action mechanisms of the yeast *Meyerozyma caribbica* for the control of the phytopathogen *Colletotrichum gloeosporioides* in mangoes. *Biological Control*. 65:293-301. doi:10.1016/j.biocontrol.2013.03.010

[16] Kancelista A, Tril U, Stempniewickz R, Piegza M, Szczech M, Witowska D. 2013. Application of lignocellulosic waste materials for the production and stabilization of *Trichoderma* biomass. Polish Journal of Environmental Studies. 22(4):1083-1090. doi:bwmeta1.element.agro-6ccb71f0-b739-49a9-a9ea-2b515c9f6d5a.

[17] Motta FL, Santana MHA. 2014. Solid-state fermentation for humic acid production by *Trichoderma reesei* strain using an oil palm empty fruit bunch as the substrate. *Applied Biochemistry and Biotechnology*. 172:2205-2217. doi:10.1007/s12010-013-0668-2.

[18] Krishna C. 2005. Solid-state fermentation an overview. *Critical Reviews in Biotechnology*. 25(1): 1-30. doi:10.1080/07388550590925383.